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Draft Genome Sequences of *Enterobacteriales* Strains Isolated from the International Space Station

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ABSTRACT

The whole-genome sequences of 26 strains isolated from the International Space Station were generated, and the strains were identified as being members of the order *Enterobacteriales*. Characterization of these whole-genome sequences might enable the identification of potential pathogenic bacteria that have been adapting to the space environment.

ANNOUNCEMENT

Members of the order *Enterobacteriales* have been found to exhibit human pathogenicity and therefore pose a health risk for people on Earth and for astronauts aboard the International Space Station (ISS) (1, 2). The latter is of concern for long-duration missions, as astronauts have been shown to be immunocompromised (3). Importantly, these bacteria are able to adapt to extreme conditions such as microgravity and radiation and thus persist, necessitating the development of appropriate countermeasures to control them. Members of the order *Enterobacteriales* that were found on ISS surfaces were *Pantoea brenneri*, *Pantoea agglomerans*, *Kalamiella piersonii*, and *Enterobacter bugandensis* (4–6). On Earth, *P. agglomerans* and *P. brenneri* were reported to have been isolated from human infections (4). *K. piersonii* is a member of a novel genus in the family *Erwiniaceae* that has exhibited resistance to multiple clinical drugs, such as penicillin and vancomycin, allowing it to be an emerging pathogen (5). *E. bugandensis* was documented from blood as a causative agent of septicemia in various geological locations (7). Analyses of draft genome assemblies for these species might pave the way to identify the genetic processes responsible for potential pathogenicity, as previously reported for some of these strains (5, 6).

The strains used for whole-genome sequencing (WGS) were isolated from four different locations in the ISS across three flights and are detailed in Table 1 (8). The ISS surface samples collected and brought back to Earth were aseptically handled, suitable aliquots of the sample concentrate (100 µl) were plated onto Reasoner's 2A (R2A) medium and incubated at 25°C for 7 days, and a single well-isolated colony was archived at −80°C until DNA extraction. DNA was extracted from cultures grown in R2A medium using the ZymoBIOMICS DNA MagBead kit according to the manufacturer's instructions.

TABLE 1.

Metadata and genome statistics for *Enterobacter*, *Kalamiella*, and *Pantoea* strains isolated from various ISS environmental surfaces during the Microbial Tracking 1 flight project

Sample name	Nearest species identity[a]	GenBank accession no.	Raw sequence accession no.	Flight(s) or facility[b]	Sampling location[c]	No. of contigs[d]	Genc size (
IF2SW-B4	*E. bugandensis*	JABWOY000000000	SRR11885007	F1-2	WHC	36	4,892
IFACSW-B2	*E. bugandensis*	JABWOX000000000	SRR11885006	F1	FC	40	4,892
IFACSW-B4	*E. bugandensis*	JABWOW000000000	SRR11885005	F1	FC	35	4,892
IFACSW-B5	*E. bugandensis*	JABWOV000000000	SRR11885004	F1	FC	37	4,891
IFACSW-P1	*E. bugandensis*	JABWOU000000000	SRR11885003	F1	FC	36	4,891
IF2SW-F2	*E. bugandensis*	JACBPD000000000	SRR12071883	F1-2	WHC	25	4,892
IF2SW-F3	*E. bugandensis*	JACBPE000000000	SRR12071879	F1-2	WHC	22	4,892
F3-6B(4)	*K. piersonii*	JACBPM000000000	SRR12071882	F3-6	PMM	39	4,850
F3-6B(5)	*K. piersonii*	JACBPN000000000	SRR12071881	F3-6	PMM	50	4,850
IIIF_BACT_A	*K. piersonii*	JACBPO000000000	SRR12071880	F3-6	PMM	42	4,849
FJII-L5-SW-P2	*P. agglomerans*	JACBPL000000000	SRR12071872	JPL SAF II	Cleanroom floor	26	4,861
IF5SW-B1	*P. brenneri*	JABWPM000000000	SRR11885013	F1-5	N1-O4	108	5,022
IF5SW-B2	*P. brenneri*	JABWPL000000000	SRR11885012	F1-5	N1-O4	107	5,023
IFACSW-B3	*P. brenneri*	JABWPK000000000	SRR11885002	F1	FC	108	5,023

Sample name	Nearest species identity[a]	GenBank accession no.	Raw sequence accession no.	Flight(s) or facility[b]	Sampling location[c]	No. of contigs[d]	Genc size (
IF5SW-P1	*P. brenneri*	JABWPJ000000000	SRR11885001	F1-5	N1-O4	106	5,023
IF5SW-P2	*P. brenneri*	JABWPI000000000	SRR11885000	F1-5	N1-O4	106	5,023
IFACSW-P2	*P. brenneri*	JABWPH000000000	SRR11884999	F1	FC	108	5,023
IIF5SW-B1	*P. brenneri*	JABWPG000000000	SRR11884998	F1-5	N1-O4	106	5,022
IIF5SW-B2	*P. brenneri*	JABWPF000000000	SRR11884997	F1-5	N1-O4	106	5,023
IIF5SW-B5	*P. brenneri*	JABWPE000000000	SRR11884996	F1-5	N1-O4	111	5,021
IIF5SW-P1	*P. brenneri*	JACBPF000000000	SRR12071878	F2-5	N1-O4	75	5,020
IIF5SW-P2	*P. brenneri*	JACBPG000000000	SRR12071877	F2-5	N1-O4	75	5,022
IIF5SW-P3	*P. brenneri*	JACBPH000000000	SRR12071876	F2-5	N1-O4	75	5,021
IIF5SW-P4	*P. brenneri*	JACBPI000000000	SRR12071875	F2-5	N1-O4	75	5,023
IIF5SW-P5	*P. brenneri*	JACBPJ000000000	SRR12071874	F2-5	N1-O4	75	5,023
IIFCSG-B1	*P. brenneri*	JACBPK000000000	SRR12071873	CRV2	CRV-FC	74	5,022

[a]The 16S rRNA gene sequences were retrieved from the whole-genome sequence of the queried genome and analyzed with BLAST against type strains for all 16S rRNA sequences in the NCBI database. The bacterial species identity was determined when the queried sequence showed >97.5% similarity to the 16S rRNA gene sequence of the type strain (*E. bugandensis* DSM 29888^T, *K. piersonii* DSM 108198^T, *P. agglomerans* DSM 3493^T, or *P. brenneri* DSM 24232^T). The whole-genome sequence of the nearest neighbor was further selected for ANI evaluation. The ANI value for all strain comparisons was 99%.

[b]Hyphenated designations indicate the flight number followed by the location; for example, F1-2 indicates flight 1 and location 2. JPL, Jet Propulsion Laboratory; SAF, Spacecraft Assembly Facility; CRV, commercial resupply vehicle.

[c]WHC, waste and hygiene compartment; PMM, permanent multipurpose module; FC, field control (a sampling wipe was exposed to the air for 120 s at the center of node 2); N1-O4, node 1 overhead 4.

[d]Contigs that were less than 200 nucleotides long were not analyzed.

WGS of 26 bacterial isolates from the ISS was performed using the Illumina Nextera Flex protocol for library preparation, as used in similar studies (6). The NovaSeq 6000 system with an S4 flow cell (paired-end 2 × 150-bp reads) was used to execute paired-end sequencing. FastQC (v0.11.7) was used to validate the quality of the raw sequencing data (9). Adapter trimming and quality filtering were carried out using the software fastp (v0.20.0) to perform quality control (10). The cleaned sequences were assembled using SPAdes (v3.11.1) (11). The N_{50} values, numbers of contigs, and total genome lengths were generated using QUAST (v5.0.2) and used to assess the quality of the final assembly (12). The average nucleotide identity (ANI) values were calculated by comparing all strains to their respective type strains, and their taxonomic affiliations and genome statistics are given in Table 1 (13). The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (v.4.11 and v.4.12) was used for genome annotation. Default parameters were used for all software.

Data availability.

This WGS project was deposited in DDBJ/ENA/GenBank (accession numbers are given in Table 1 [BioProject accession no. PRJNA635942]) and also deposited in the NASA GeneLab database (accession no. GLDS-302 and GLDS-311). The versions described in this paper are the first versions.

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Associated Data

This section collects any data citations, data availability statements, or supplementary materials included in this article.

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